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Sodium nitroprusside, ferricyanide, nitrite and nitrate decrease the thermo-dormancy of lettuce seed germination in a nitric oxide-dependent manner in light

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Abstract

Lettuce (*L. sativa* L. 'Jianye Xianfeng No. 1') seeds can exhibit both photodormancy and thermodormancy (thermoinhibition); however, the mechanism for thermoinhibition of seed germination is poorly understood. Here, we have investigated the response of lettuce seed germination to temperature and light, the effect of sodium nitroprusside (SNP), potassium ferricyanide (Fe(III)CN), nitrite, nitrate and NO scavenger 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO) on the thermoinhibition of seed germination, and the inhibiting role of PTIO on SNP, Fe (III)CN, and acidified nitrite to determine the mechanisms of thermoinhibition of seed germination. Our data showed that the optimal temperature for Jianye Xianfeng No. 1 lettuce seed germination was 13–15 °C in darkness, and 11–19 °C in light. The thermoinhibition of seed germination could be decreased by SNP, Fe(III)CN, nitrite and nitrate in light and by nitrite and nitrate in darkness. Changes in lettuce seed germination caused by PTIO at 1–200 μM were not observed at the optimal temperatures. Germination was increased by PTIO at 1–100 μM and was decreased by PTIO at 200 μM in light at 23 °C, and that was gradually decreased by 1–200 μM in darkness at 17 °C. In light, SNP, Fe(III)CN and vapours produced by SNP, Fe(III)CN and acidified nitrite could effectively decrease the thermoinhibition of seed germination, and these promoting effects were inhibited by PTIO at 200 μM. In darkness, nitrate and nitrite at 5 and 10 mM plus PTIO stimulated the germination of seeds. Our data show that thermoinhibition of lettuce seed germination is temperature- and light-dependent, was decreased by SNP, Fe(III)CN, nitrite and nitrate in a nitric oxide-dependent manner in light.

Keywords: *Lactuca sativa*; Nitric oxide; PTIO; SNP; Thermoinhibition of seed germination

1. Introduction

Temperature is a primary factor regulating seed germination either directly, through action on germination itself, or indirectly, by affecting dormancy and viability. The suppression of germination at supraoptimal temperatures is called thermoinhibition, and seeds that have become dormant by exposure to high temperatures are said to be in thermodormancy (Negm et al., 1972; Vidaver and Hsiao, 1975). Thermoinhibition plays an ecologically important role in the detection of the appropriate seasonal timing for germination in soil-buried seeds of winter annual plants (Baskin and Baskin, 1998), and it often causes delayed or poor germination of cultivated crops, flowers, and vegetables that have relatively low optimal temperatures for seed germination.

Lettuce (*Lactuca sativa*) seeds can exhibit thermoinhibition and do not germinate in darkness and even in light at 26 °C or above, depending on variety (Black et al., 2006). Several factors influence the upper temperature limit for lettuce seed germination (Nambara et al., 2010). Seeds matured under warm temperatures have higher germination temperature limits than do those matured

Abbreviations: ABA, Abscisic acid; c-PTIO, 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3 oxide; GA, Gibberellin; NO, Nitric oxide; PBN, N-tert-butyl-α-phenylnitrone; PTIO, 2-Phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide; Fe(III)CN, Potassium ferricyanide; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SNP, Sodium nitroprusside.

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under cooler temperatures (Kozarewa et al., 2006). Exogenous application of ethylene, GA, and red light can increase the upper temperature limit of lettuce seed germination, and ABA can decrease it (Saini et al., 1986, 1989; Dutta and Bradford, 1994; Kristie and Fielding, 1994; Gonai et al., 2004). Higher temperatures increase the sensitivity of lettuce seed germination to inhibition by ABA (Roth-Bejerano et al., 1999; Argyris et al., 2008), stimulate ABA synthesis and repress GA synthesis and signaling through the action of ABA in *Arabidopsis thaliana* seeds (Toh et al., 2008).

Nitric oxide (NO) is a reactive, gaseous, free radical that functions as a potent signaling molecule in plant and animals. It has been proposed that NO promotes the germination of seeds, either by reducing seed dormancy or by minimizing the effects of environmental conditions that inhibit germination (Bethke et al., 2007). It has been shown that NO donor sodium nitroprusside (SNP) could lessen or remove dormancy from *A. thaliana* (Bethke et al., 2004, 2006a, b, 2007), barley (*Hordeum vugare*) (Bethke et al., 2004), switchgrass (*Panicum virgatum*), big bluestem (*Poa ampla*) and Indiangrass seeds (Sarath et al., 2006) in light, but not in the dark. In contrast to results mentioned above, Beligni and Lamattina (2000) reported that two NO donors, SNP and *S*-nitroso-*N*-acetylpecicillamine (SNAP) induced the germination of lettuce (*L. sativa* L. 'Grand Rapids') seeds at 26 °C in complete darkness. Seeds of *A. thaliana* and barley have non-deep physiological dormancy, whether seed germination in light or dark is a germination requirement, i.e. the response to light/darkness is shown after physiological dormancy is broken (Bethke et al., 2004). However, it is unknown whether NO donor compounds can decrease thermoinhibition of lettuce seed germination at superoptimal temperatures in light or darkness. Bethke et al. (2006a, b) have shown that SNP, cyanide, nitrite and nitrate break seed dormancy of *A. thaliana* in a nitric oxide-dependent manner. It is not clear whether these compounds have a similar role at superoptimal temperatures in light or darkness in decreasing thermoinhibition of lettuce seed germination.

In the present paper, mature lettuce (*L. sativa* L. 'Jianye Xianfeng No. 1') seeds were used as experimental material. We investigated (1) the response of lettuce seed germination to temperature and light, (2) the effect of SNP, potassium ferricyanide (Fe(III)CN), nitrite, nitrate and NO scavenger PTIO on the thermoinhibition of seed germination, and (3) the inhibiting role of PTIO on SNP, Fe(III)CN, and acidified nitrite to determine whether these compounds decrease the thermoinhibition of seed germination in a NO-dependent manner.

2. Materials and methods

2.1. Plant material

Lettuce (*L. sativa* L. cv. Jianye Xianfeng No. 1) seeds were kindly provided by Zhongdu Sichuan Co. Ltd (Chengdu, Sichuan, China). Water content and germination of seeds were 8.39 ± 0.35% and ≥ 90%, respectively. The seeds were stored in the aluminum foil bag at −20 °C until used in the experiments.

2.2. Germination testing

Three replicates of 50 seeds each were germinated on two layers of filter paper moistened with 4 ml of distilled water or different treatment solutions (SNP, Fe(III)CN, nitrite, nitrate and PTIO) in closed 90-mm diameter Petri dishes at different temperatures in light (an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark) or in continuous dark for 72 h. A weak green safe-light was used for manipulation of seeds in darkness. A radicle length of 2 mm was used as the criterion for germination.

For effect of vapours evolved from different treatment solutions (SNP, Fe(III)CN, acidified nitrite and nitrate) on seed germination, lettuce seeds were incubated in open 55-mm diameter Petri dishes, referred to as receiver dishes, containing 4 ml of distilled water or of PTIO at 200 μM. Three receiver dishes and one donor dishes which contain 10 ml of different treatment solutions (illustrated in Fig. 7c), were enclosed in a sealed 150-mm Petri dishes, so that vapours evolved from the donor dishes could be transferred to the receiver dishes. These seeds were incubated in light (as described above) at 23 °C for 3 days.

2.3. Chemicals

Sodium nitroprusside and PTIO were purchased from Sigma-Aldrich Co., the others from Beijing Chemical Co. Ltd, Beijing, China. PTIO was dissolved in distilled water and stored at −20 °C. Nitrite, nitrate, SNP and Fe(III)CN were freshly prepared, respectively, before experiments were carried out.

2.4. Statistical analysis

All data were analysed using a one-way ANOVA model from the SPSS 13.0 package for Windows, the significance of mean differences was determined using the Student–Newman–Keulsl (S–N–K, $P = 0.05$) (SPSS Inc. 2008). To stabilize the variances, all data were arcsine-transformed prior to statistical analysis.

3. Results

3.1. Germination of lettuce seeds in response to temperature and light

The germination of lettuce seeds was obviously influenced by temperatures and light conditions (P value ≤ 0.001, Fig. 1). In darkness, the optimal temperature for lettuce seed germination was 13–15 °C, the germination percentage of seeds was zero at 21 °C. However, light could decrease effectively the thermoinhibition of lettuce seed germination. In light, the optimal temperature for seed germination was 11–19 °C, the thermoinhibition of seed germination increased at temperatures above 19 °C, and the germination percentage of seeds was zero at 27 °C (Fig. 1). It was noted that the optimal temperature range

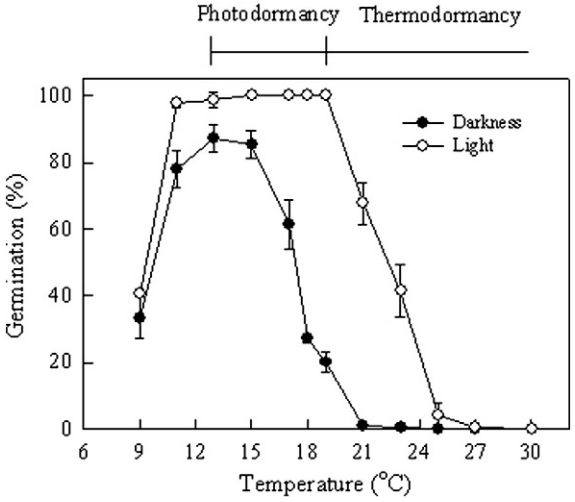

Fig. 1. Response of lettuce seed germination to temperature and light (an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark). Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each.

of seed germination was wider and higher in light than in darkness, and that germination percentage of seeds was higher at same temperatures in light than in darkness (Fig. 1). For Jianye Xianfeng No. 1 lettuce seeds, it appears that the temperature range of photodormancy was 13–19 °C, and temperature of thermoinhibition was above 19 °C (Fig. 1).

After incubation at 25–35 °C in light for 3 days, the non-germinated seeds were continuously incubated at 10 °C in light for 3 days, and then were transferred to a fluctuating temperature (33/23 °C) to grow for 3 days. One-hundred percent recovery of germination and above 94% recovery of emergence of non-germinated seeds have been observed (Table 1, P value = 0.113), showing further that germination of these seeds was thermoinhibited at 25–35 °C.

Table 1
Recovery of thermoinhibition of lettuce seed germination.

Germination temperature (°C)	Germination (%)	Recovery of germination [a] (%)	Recovery of emergence [b] (%)
25	4 ± 1	100.0 ± 0.0A	100.0 ± 0.0A
27	0	100.0 ± 0.0A	98.0 ± 0.0A
30	0	100.0 ± 0.0A	94.0 ± 5.3A
33	0	100.0 ± 0.0A	94.0 ± 2.0A
35	0	100.0 ± 0.0A	95.3 ± 3.4A

[a] After incubation at different temperatures mentioned in table and an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark for 3 d, the non-germinated seeds were continuously germinated for 3 d at 10 °C and an alternating photoperiod. Radicle protrusion for 2 mm was used as the criterion for completion of germination.

[b] After recovery-germination at 10 °C and an alternating photoperiod for 3 d, the seedlings were transferred to a fluctuating temperature (33 °C, light, 14 h/23 °C, dark, 10 h) and an alternating photoperiod to grow for 3 d. Radicle length for 1 cm and shoot length for 5 mm were used as the criterion for normal seedling. Emergence percentage = number of normal seedlings/number of tested seeds. All values are means ± SD of three replicates of 50 seeds each. Numbers in a column followed by different uppercase letters are significantly different from each other (S–N–K, P = 0.05).

3.2. SNP and Fe(III)CN decrease the thermoinhibition of lettuce seed germination at different temperatures and light conditions

To investigate whether SNP and Fe(III)CN could decrease the thermoinhibition of seed germination in different temperatures and light conditions, lettuce seeds were germinated in 1–500 μM SNP or 10–1000 μM Fe(III)CN at 21, 23, 25 °C in light or at 18, 21 °C in darkness. It was observed that in light, SNP at 1–300 μM could markedly decrease thermoinhibition of seed germination (P value ≤ 0.001), but these effects were temperature-dependent; for example, the optimal concentration of SNP for seed germination was 10–300 μM at 21 °C, was 100 μM at 23 °C, was 300 μM at 25 °C (Fig. 2a). However, in darkness, no germination was promoted by SNP at 1–300 μM at 18–21 °C (data not shown).

At 21 and 23 °C in light, the thermoinhibition of lettuce seed germination was decreased by Fe(III)CN at 10–100 μM, and was increased by Fe(III)CN above 300 μM (P value ≤ 0.001,

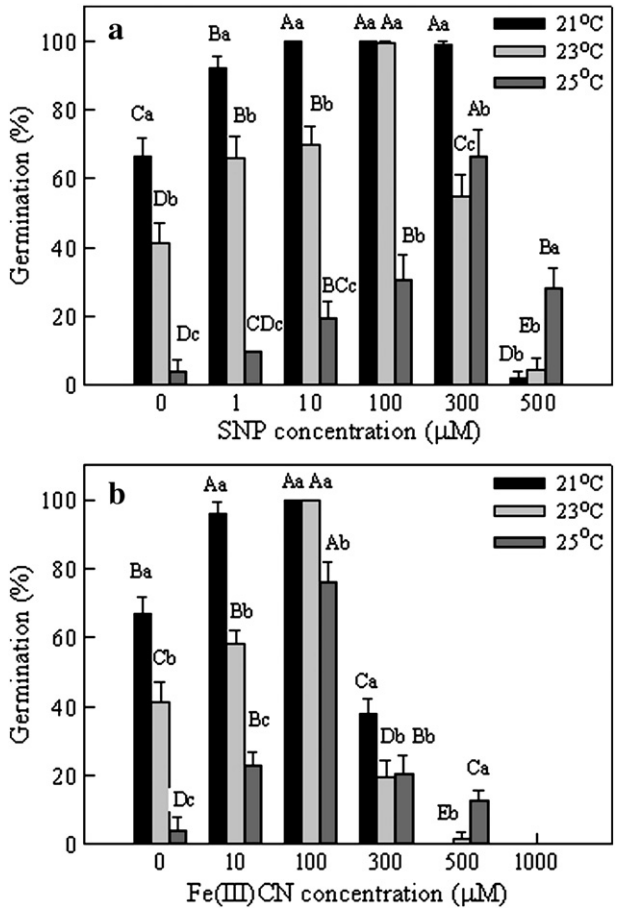

Fig. 2. SNP (a) and Fe(III)CN (b) stimulate germination of lettuce seeds at 21, 23 and 25 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark. Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each. Bars with different uppercase letters are significantly different within seeds germinating at same temperature and different concentrations of SNP or Fe(III)CN, and those with different lowercase letters are significantly different within seeds germinating at same concentration of SNP or Fe(III)CN and different temperatures (S–N–K, P = 0.05).

Fig. 2b). However, at 25 °C in light, the thermoinhibition was decreased by Fe(III)CN at 10–500 μM, and was increased by Fe (III)CN at 1000 μM (P value ≤ 0.001, Fig. 2b). The optimal concentration of Fe(III)CN also was temperature-dependent in decreasing thermoinhibition of lettuce seed germination. For seed germination at 21, 23 and 25 °C in light, the optimal concentration of Fe(III)CN was 100 μM (Fig. 2b). In darkness, no germination of seeds was promoted by Fe(III)CN at 10–1000 μM at 18–21 °C (data not shown).

3.3. Nitrite and nitrate decrease the thermoinhibition of lettuce seed germination at different temperatures and light conditions

It has been reported that no germination of lettuce seeds was promoted by nitrite and nitrate at 100 μM at 26 °C under complete darkness (Beligni and Lamattina, 2000). To determine the possible effect of nitrite and nitrate on germination, lettuce seeds were germinated in 5–100 mM nitrite and nitrate at different temperatures in light or in darkness. The results showed that at 21, 23 and 25 °C in light, thermoinhibition of seed germination was decreased by nitrite at 5–50 mM, and was increased by nitrite at 80–100 mM (P value ≤ 0.001, Fig. 3a). In darkness, thermoinhibition of seed germination was decreased by nitrite at 5–80 mM, and was increased by nitrite at 100 mM at 18 (P value ≤ 0.001) and 21 °C (P value = 0.003) (Fig. 3b).

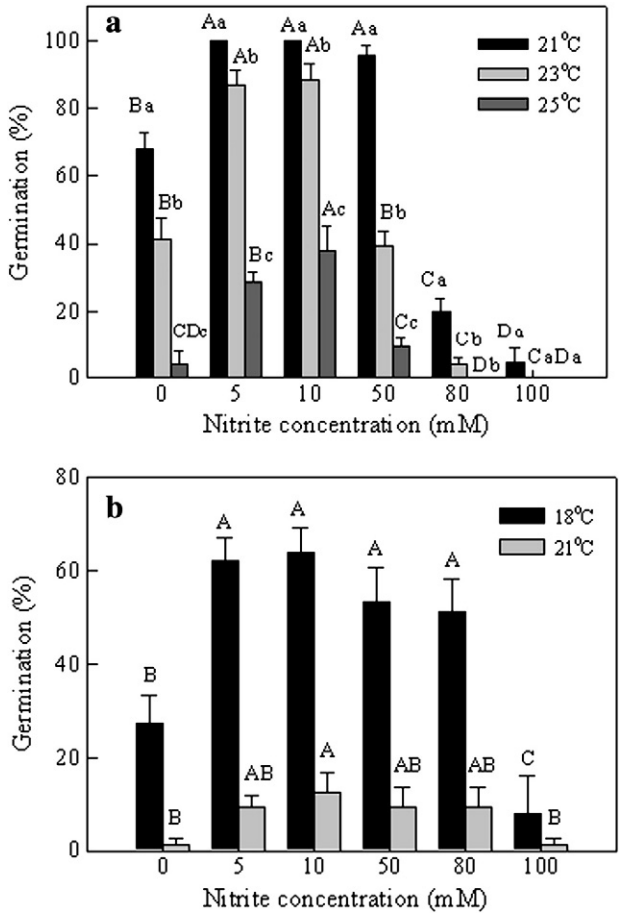

Fig. 3. Nitrite stimulates germination of lettuce seeds at 21, 23 and 25 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark (a) or at 18 and 21 °C in darkness (b). Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each. Bars with different uppercase letters are significantly different within seeds germinating at same temperature and different concentrations of nitrite, and those with different lowercase letters are significantly different within seeds germinating at same concentration of nitrite and different temperatures (S–N–K, P = 0.05).

In light, thermoinhibition of seed germination was decreased by nitrate at 5–50 mM, and it was increased by nitrate at 80–100 mM at 21 °C (P value ≤ 0.001). It was decreased by nitrate at 5–50 mM, increased by nitrate at 100 mM at 23 °C (P value = 0.001), and decreased by nitrate at 5 and 10 mM at 25 °C (P value ≤ 0.001) (Fig. 4a). However, in darkness, thermoinhibition of seed germination was decreased by nitrate at 10 mM, and it was increased by nitrate at 100 mM at 18 °C (P value ≤ 0.001). No germination of seeds was obviously promoted by nitrate at 10 –100 mM at 21 °C (Fig. 4b).

The promotion of seed germination by nitrite and nitrate was higher in light than in darkness (Figs. 3 and 4). Thermoinhibition of seed germination was much higher in nitrite than in nitrate at 80–100 mM at 21, 23 and 25 °C in light, and it was much lower in nitrite than in nitrate at 5–50 mM in darkness at 18 °C (Figs. 3 and 4).

3.4. Effects of NO scavenger PTIO on germination of lettuce seeds

To test whether NO scavenger PTIO inhibits germination of lettuce seeds, seeds were incubated in different concentrations of PTIO at different temperatures and light conditions. No change in seed germination was observed in PTIO at 1–200 μM at 19 °C in light (P value = 0.400) or at 13 °C in darkness (P value = 0.984), which were the optimal conditions for germination of lettuce seeds, respectively (Fig. 5). However, seed germination was stimulated by PTIO at 1–100 μM and inhibited by PTIO at 200 μM at 23 °C in light (P value ≤ 0.001), and it was inhibited by PTIO at 1–200 μM at 17 °C in darkness (P value = 0.02) (Fig. 5).

3.5. NO scavenger PTIO affects the effect of SNP, Fe(III)CN, nitrate and nitrite in germination of lettuce seeds

At 23 °C in light, SNP at 100 and 300 μM, Fe(III)CN at 10 and 100 μM, nitrate and nitrite at 5 and 10 mM increased the germination of lettuce seeds (P value ≤ 0.001), but these promoting effects could be inhibited by PTIO at 200 μM (Fig. 6a, b).

At 17 °C in darkness, germination of lettuce seeds was not stimulated by nitrate at 5 and 10 mM, and it was stimulated by nitrite at 5 and 10 mM (P value ≤ 0.001) (Fig. 6c). However, at 17 °C in darkness, PTIO at 200 mM did not inhibit germination and, in contrast, increased the promoting effect of nitrate and nitrite at 5 and 10 mM on seed germination (P value ≤ 0.001) (Fig. 6c).

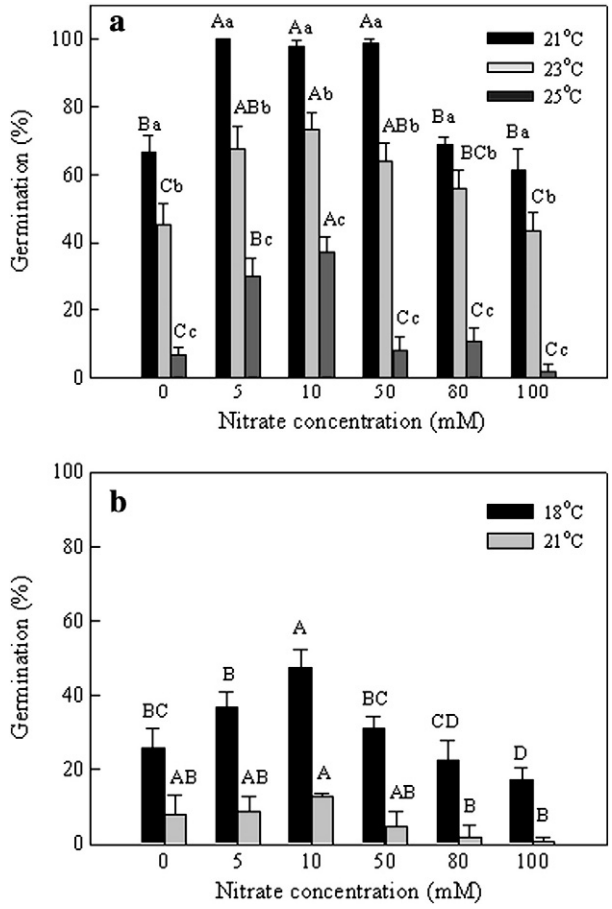

Fig. 4. Nitrate stimulates the germination of lettuce seeds at 21, 23 and 25 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark (a) or at 18 and 21 °C in darkness (b). Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each. Bars with different uppercase letters are significantly different within seeds germinating at same temperature and different concentrations of nitrate, and those with different lowercase letters are significantly different within seeds germinating at same concentration of nitrate and different temperatures (S–N–K, $P = 0.05$).

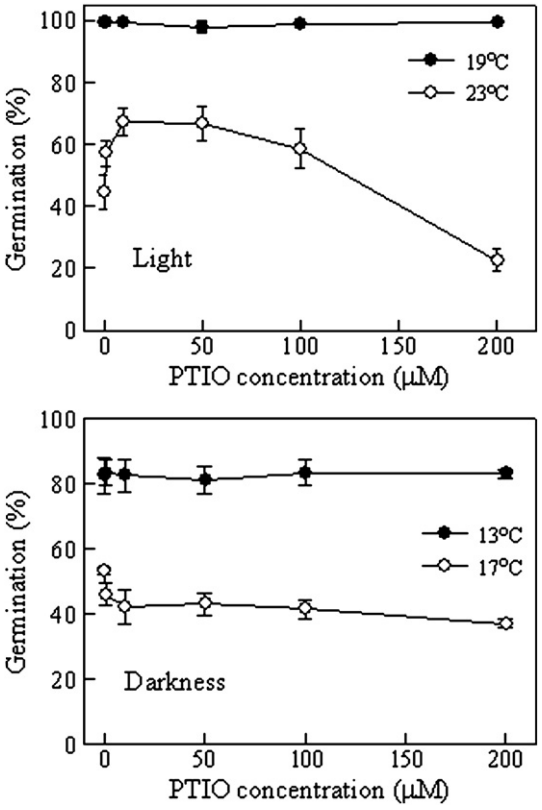

Fig. 5. Effect of PTIO on germination of lettuce seeds at 19 and 23 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark or at 13 and 17 °C in darkness. Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each.

3.6. Gases evolved from SNP, Fe(III)CN and acidified nitrite decrease thermoinhibition of lettuce seed germination and PTIO inhibits the promoting effect of these gases

To determine the effect of vapours produced by SNP, Fe(III)CN, nitrite, nitrate and acidified nitrite and nitrate on germination, we have evaluated the germination response of seeds by exposing them to vapours from donor dishes (as described in Fig. 7c). The vapours produced by SNP and Fe(III)CN at 100 μM increased the germination percentage of seeds (P value ≤ 0.001), but those produced by nitrite and nitrate at 5 mM had no promoting effect on seed germination. PTIO at 200 μM obviously inhibited both the promoting effects of SNP and Fe(III)CN and the germination of seeds which were subjected to vapours produced by nitrite and nitrate at 5 mM (Fig. 7a).

Seed germination was stimulated by the vapours produced by acidified nitrite at 0.5–5 mM, and it was inhibited by acidified nitrite at 10 mM (P value ≤ 0.001). The optimal concentration of acidified nitrite was 1–5 mM (Fig. 7b). PTIO at 200 μM also could effectively inhibit the promoting effect of vapours produced by acidified nitrite, but these inhibitory effects were acidified-nitrite-concentration-dependent, i.e. could be partially overcome by high concentrations of acidified-nitrite vapour (Fig. 7b). The vapours from acidified nitrate, and those from HCl and KCl did not stimulate seed germination as compared with the control (water); however, PTIO at 200 μM obviously inhibited germination of seeds treated by vapours from acidified nitrate at 5 mM (Fig. 7b).

4. Discussion

The germination percentage of Jianye Xianfeng No. 1 lettuce seeds was inhibited by temperatures above 15 °C in darkness and by temperatures above 19 °C in light (Fig. 1). The optimal temperature for lettuce seed germination was much higher in light than in darkness (Fig. 1). The non-germinated seeds at 25–35 °C in light for 3 days could recover to germinate at 10 °C in light (Table 1). These results, taken together, showed that the germination of Jianye Xianfeng No. 1 lettuce seeds is both photodormant and thermoinhibited (thermodormant). The temperature range of photodormancy was 13–19 °C, and the temperature for thermoinhibition was above 19 °C (Fig. 1). This is first clear report of the temperature range for the photodormancy and of thermoinhibition of lettuce seed germination. However,

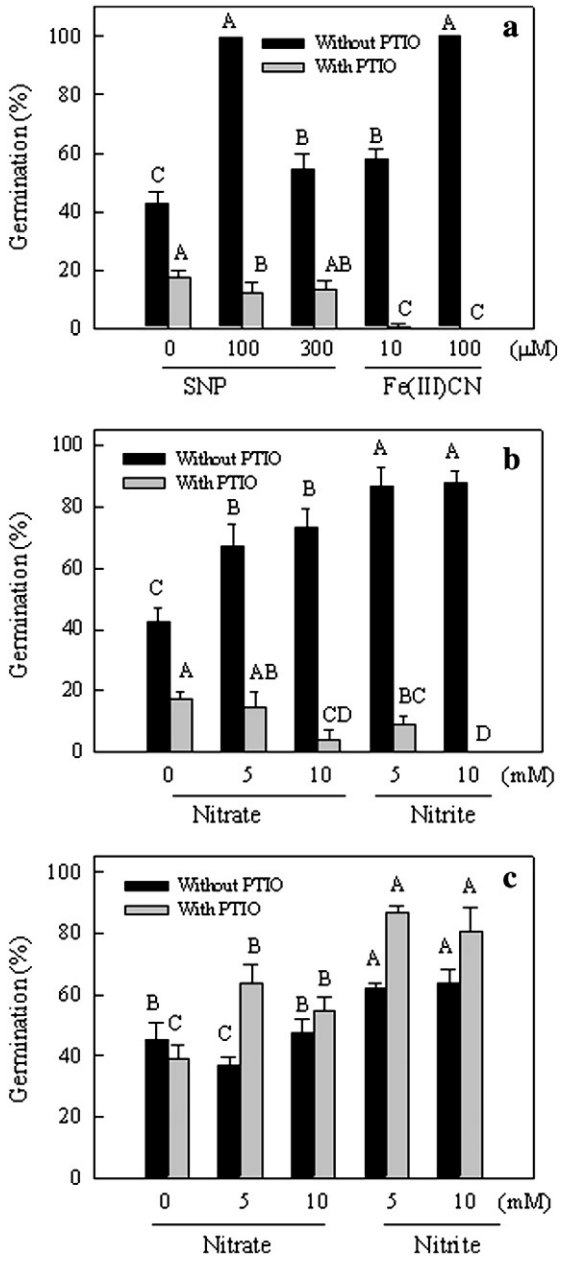

Fig. 6. Effect of PTIO on SNP, Fe(III)CN, nitrate and nitrite in germination of lettuce seeds at 23 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark (a, b) or at 17 °C in darkness (c). Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each. Bars with different uppercase letters are significantly different within seeds germinating at different treatments (S–N–K, $P = 0.05$).

photodormancy and thermoinhibition of seed germination are variety-dependent (Black et al., 2006) and may have different mechanisms.

At temperatures above 25 °C, germination of Grand Rapids lettuce seeds occurs only in light, being a phytochrome-dependent process (Bewley and Black, 1982). Beligni and Lamattina (2000) showed that at 26 °C in darkness, SNP at 100 μM stimulates significantly the germination of Grand Rapids lettuce seeds. In contrast, our results showed that at 18–21 °C in darkness, no germination of Jianye Xianfeng No. 1 lettuce seeds was promoted by SNP at 1–300 μM (data not shown). We consider that Grand Rapids lettuce seeds are photodormant at 26 °C in darkness, while Jianye Xianfeng No. 1 lettuce seeds are thermoinhibited at 18–21 °C in darkness, therefore, they have different responses to SNP at different temperatures. Giba et al. (1998), working with photodormant seeds of the Empress tree (*Paulownia tomentosa*), showed that the red light requirement for germination could be reduced by treatment with NO donors SNP. Light and van Staden (2003) did not observe increased germination of Grand Rapids lettuce seeds imbibed in the dark with SNP or PBN, and they suggested that it could be attributed to differences in seed batches. The thermoinhibition of lettuce seed germination was notably decreased by SNP at 1–300 μM at 21, 23 and 25 °C in light (Fig. 2a) and by vapours produced by SNP at 100 μM at 23 °C in light (Fig. 7a). These results were similar to those of Bethke et al. (2004, 2006a, b, 2007) and Sarath et al. (2006) who found that the NO donor SNP could break the dormancy of *A. thaliana*, barley and switchgrass seeds.

The thermoinhibition of lettuce seed germination was decreased by Fe(III)CN at 10 and 100 μM and increased by Fe(III)CN at 300–1000 μM at 21 and 23 °C in light (Fig. 2b), and it was decreased by vapours produced by Fe(III)CN at 100 μM at 23 °C in light (Fig. 7a). These results were in accordance with those of Bethke et al. (2004, 2006a, b). However, no germination of lettuce seeds was promoted by Fe(III)CN at 10–1000 μM at 18–21 °C in darkness (data not shown). The decomposition of SNP and Fe(III)CN is light-dependent (Feelisch, 1998; Kozlik et al., 2004). The decomposition of SNP results in the production of NO and cyanide, and Fe(III)CN and free cyanide (Meeussen et al., 1992). It is clear from our results (Fig. 2) that Fe(III)CN is as effective as SNP in decreasing the thermoinhibition of lettuce seed germination. Bethke et al. (2006a) proposed that NO in seeds is enzymatically and non-enzymatically catabolized. *A. thaliana* plants contain non-legume haemoglobin, which converts NO to nitrate. Applied CN^- might bind to haemoglobin and prevent haemoglobin-dependent detoxification of NO, thereby bringing about an increase in NO concentration within the seed (Dordas et al., 2003). Cytochrome *c* oxidase has been shown to metabolize NO in mammals (Torres et al., 2000; Giuffre et al., 2005) and binding of CN^- to cytochrome *c* oxidase strongly inhibited NO catabolism by this enzyme (Borutaite and Brown, 1996).

No germination of lettuce seeds was observed in nitrite and nitrate at 100 μM at 26 °C under complete darkness (Beligni and Lamattina, 2000). Thermoinhibition of lettuce seed germination was decreased by nitrite and nitrate in a dose-dependent manner either at 21, 23 and 25 °C in light or at 18 and 21 °C in darkness (Figs. 3 and 4). It has been observed that the promotion of seed germination by nitrite and nitrate was higher in light than in darkness. Thermoinhibition of seed germination was much higher in nitrite than in nitrate at 80–100 mM at 21, 23 and 25 °C in light, and it was much lower in nitrite than in nitrate at 5–50 mM at 18 °C in darkness (Figs. 3 and 4). These results were consistent with those for *A. thaliana* by Bethke et al. (2004, 2006a, b). Nitrate and nitrite have often been shown

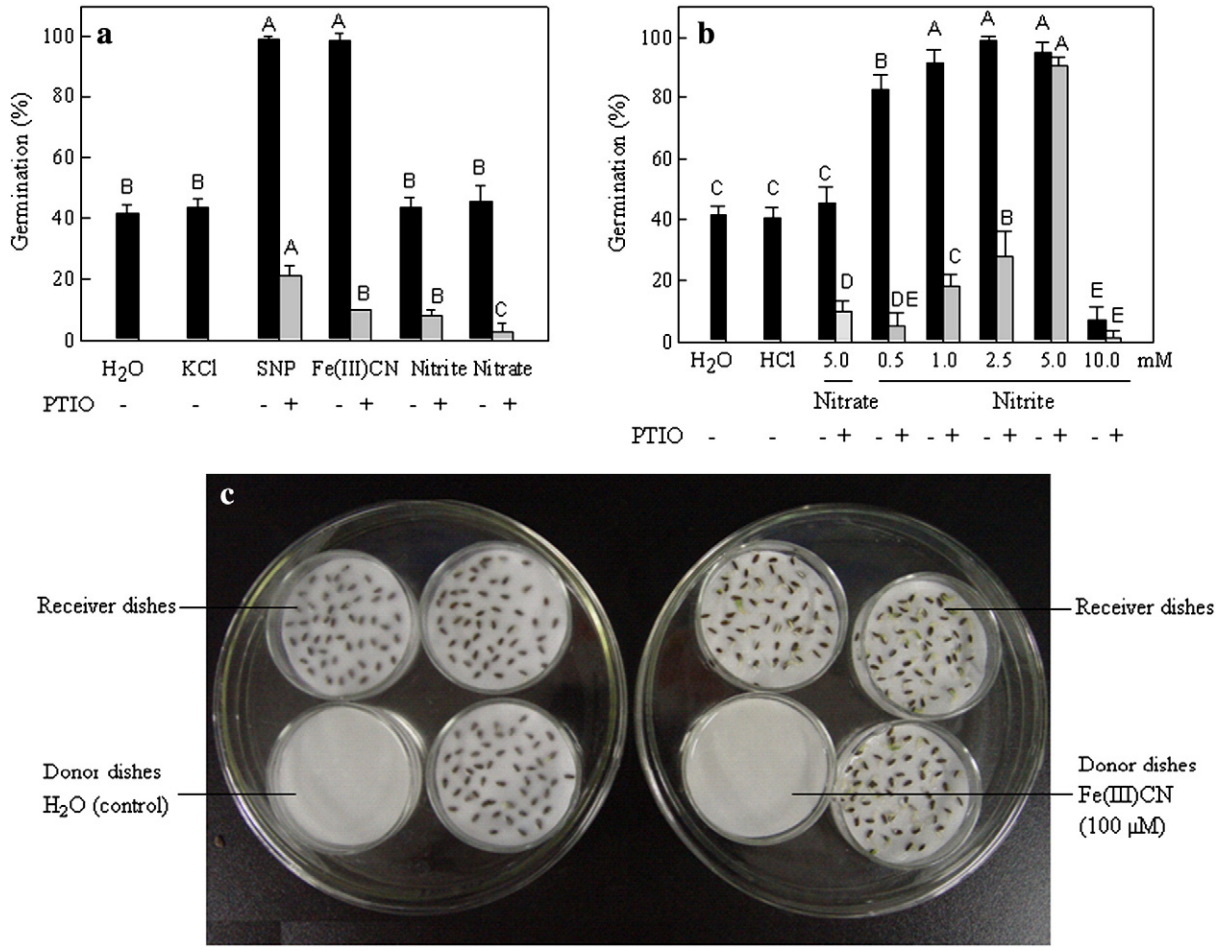

Fig. 7. Effect of vapours evolved from SNP (100 μM), Fe(III)CN (100 μM), nitrite (5 mM), nitrate (5 mM) (a), and acidified nitrate and nitrite (b) on germination of lettuce seeds at 23 °C in an alternating photoperiod with 14 h light (66.3 μmol m^{-2} s^{-1}) and 10 h dark. An illustration of the experimental system used to apply vapours from different treatment solutions in a donor dishes to seeds in receiver dishes, and the photos showing that vapours evolved from Fe(III)CN at 100 μM stimulated the seed germination (c). Radicle protrusion for 2 mm was used as the criterion for completion of germination. All values are means ± SD of three replicates of 50 seeds each. Bars with different uppercase letters are significantly different within seeds germinating at different treatments (S–N–K, P = 0.05).

to break seed dormancy (Bewley and Black, 1994), and their stimulatory effects may be mediated via NO. Nitrate reductase can produce NO from NO_3^- or NO_2^- (Besson-Bard et al. 2008).

The germination conditions at 19 °C in light or at 13 °C in darkness were optimal for germination of lettuce seeds, respectively (Fig. 1), i.e. the germination of lettuce seeds was not inhibited by PTIO at 1–200 μM, indicating that other than NO, some unknown components/processes produced in optimal temperatures stimulate seed germination. These results were similar to those of Bethke et al. (2004, 2006a, b) and Liu et al. (2009) who found that NO scavenger c-PTIO did not inhibit the germination of non-dormant *A. thaliana* seeds. In contrast, c-PTIO strengthened the dormancy of dormant *A. thaliana* seeds (Bethke et al., 2004, 2006a, b; Liu et al., 2009). Interestingly, the germination of lettuce seeds was stimulated by PTIO at 1–100 μM and was inhibited by PTIO at 200 μM at 23 °C in light, and it was inhibited by PTIO at 1–200 μM at 17 °C in darkness (Fig. 5); the reasons are unknown.

At 23 °C in light, the germination of lettuce seeds was markedly promoted by SNP at 100 and 300 μM and by Fe(III) CN at 10 and 100 μM and by vapours produced by SNP and Fe (III)CN at 100 μM, but these promoting effects could be inhibited by 200 μM PTIO (Figs. 6a, 7a). The germination of lettuce seeds was stimulated by nitrate and nitrite at 5 and 10 mM (Fig. 6b), but it was not stimulated by vapours produced by nitrite and nitrate at 5 mM and KCl at 10 mM (Fig. 7a). HCl at 100 mM and acidified nitrate at 5 mM did not stimulate germination, but acidified nitrite at 0.5–5 mM stimulated the germination of lettuce seeds (Fig. 7b). However, the promoting effects of lettuce seed germination by nitrite and nitrate (Fig. 6b) and by vapour produced by acidified nitrite (Fig. 7b) and from nitrite and nitrate (Fig. 7a) were inhibited by 200 μM PTIO. These results were similar to those for *A. thaliana* (Bethke et al., 2004, 2006a, b, 2007), barley (Bethke et al., 2004) and switchgrass (Sarath et al., 2006) seeds. These data, taken together, suggest that NO is required to promote the germination of lettuce seeds caused by SNP, Fe(III)CN, nitrite and nitrate. At 17 °C in darkness, germination of lettuce seeds was not stimulated by nitrate at 5 and 10 mM, and it was stimulated by nitrite at 5 and 10 mM. On the other hand, nitrate and nitrite at 5 and 10 mM plus PTIO at 200 mM increased the germination of lettuce seeds (Fig. 6c). Akaike and Maeda

(1996) have proposed that the reaction of NO with PTIO can generate NO_2. Whether NO_2 can be produced and the NO_2 can promote seed germination is unknown here.

In conclusion, germination of Jianye Xianfeng No. 1 lettuce seeds is temperature- and light-dependent, and thermoinhibition of seed germination was decreased by SNP, Fe(III)CN, nitrite and nitrate in a nitric oxide-dependent manner in light.

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References

Akaike, T., Maeda, H., 1996. Quantitation of nitric oxide using 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO). Methods in Enzymology 268, 211–221.

Argyris, J., Dahal, P., Hayashi, E., Still, D.W., Bradford, K., 2008. Genetic variation for lettuce seed thermoinhibition is associated with temperature-sensitive expression of abscisic acid, gibberellin, and ethylene biosynthesis, metabolism, and response genes. Plant Physiology 148, 926–947.

Baskin, J.M., Baskin, C.C., 1998. Seed – Ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego.

Beligni, M.V., Lamattina, L., 2000. Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210, 215–221.

Besson-Bard, A., Pugin, A., Wendehenne, D., 2008. New insight into nitric oxide signaling in plant. Annual Review of Plant Biology 59, 21–39.

Bethke, P.C., Gubler, F., Jacobsen, J.V., Jones, R.L., 2004. Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. Planta 219, 847–855.

Bethke, P.C., Libourel, I.G.L., Jones, R.L., 2006a. Nitric oxide reduces seed dormancy in *Arabidopsis*. Journal of Experimental Botany 57, 517–526.

Bethke, P.C., Libourel, I.G.L., Jones, R.L., 2007. Nitric oxide in seed dormancy and germination. In: Bradford, K.J., Nonogaki, H. (Eds.), Seed development, dormancy and germination. Blackwell Publishing Ltd, Oxford, pp. 153–175.

Bethke, P.C., Libourel, I.G.L., Reinöhl, V., Jones, R.L., 2006b. Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. Planta 223, 805–812.

Bewley, J.D., Black, M., 1982. Physiology and biochemistry of seeds in relation to germination, vol 2. Springer-Verlag, Berlin, pp. 126–198.

Bewley, J.D., Black, M., 1994. Seeds. Physiology of development and germination, 2nd ed. Plenum Press, New York.

Black, M., Bewley, J.D., Halmer, P., 2006. The Encyclopedia of seeds. Science, technology and uses. CAB International, Oxfordshire, pp. 377–379.

Borutaite, V., Brown, G., 1996. Rapid reduction of nitric oxide by mitochondria, and reversible inhibition of mitochondrial respiration by nitric oxide. The Biochemical Journal 315, 295–299.

Dordas, C., Rivoal, J., Hill, R.D., 2003. Plant haemoglobins, nitric oxide and hypoxic stress. Annals of Botany 91, 173–178.

Dutta, S., Bradford, K.J., 1994. Water relations of lettuce seed thermoinhibition. II. Ethylene and endosperm effects on base water potential. Seed Science Research 4, 11–18.

Feelisch, M., 1998. The use of nitric oxide donors in pharmacological studies. Naunyn-Schmiedebergs Archives of Pharmacology 358, 113–122.

Giba, Z., Grubisic, D., Todorovic, S., Sajc, L., Stojakovic, D., Konjevic, T., 1998. Effect of nitric oxide-releasing compounds on phytochrome-controlled germination of Empress tree seeds. Plant Growth Regulation 26, 175–181.

Giuffre, A., Forte, E., Brunori, M., Sarti, P., 2005. Nitric oxide, cytochrome *c*oxidase and myoglobin: competition and reaction pathways. FEBS Letters 579, 2528–2532.

Gonai, T., Kawahara, S., Tougou, M., Satoh, S., Hashiba, T., Hirai, N., Kawaide, H., Kamiya, Y., Yoshioka, T., 2004. Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. Journal of Experimental Botany 55, 111–118.

Kozarewa, I., Cantliffe, D.J., Nagata, R.T., Stoffella, P.J., 2006. High maturation temperature of lettuce seeds during development increased ethylene production and germination at elevated temperatures. Journal of the American Society for Horticultural Science 131, 564–570.

Kozlik, P., Baehler, M., Puentener, C., Zerbe, O., Bigler, L., Altorfer, H.R., 2004. N-nitrosylation potential of mono-N-desethylamiodarone at physiological pH. Journal of Pharmaceutical and Biomedical Analysis 34, 399–407.

Kristie, D.N., Fielding, A., 1994. Influence of temperature on the Pfr level required for germination in lettuce cv Grand Rapids. Seed Science Research 4, 19–25.

Light, M.E., Van Staden, J., 2003. The nitric oxide-specific scavenger carboxy-PTIO does not inhibit smoke-stimulated germination of Grand Rapids lettuce seeds. South African Journal of Botany 69, 217–219.

Liu, Y., Shi, L., Ye, N., Liu, R., Jia, W., Zhang, J., 2009. Nitric oxide-induced rapid decrease of abscisic acid concentration is required in breaking seed dormancy in *Arabidopsis*. The New Phytologist 183, 1030–1042.

Meeussen, J.C.L., Keizer, M.G., van Riemsdijk, W.H., De Hann, F.A.M., 1992. Dissolution behavior of iron cyanide (*Prussian blue*) in contaminated soils. Environmental Science and Technology 26, 1832–1838.

Nambara, E., Okamoto, M., Tatematsu, K., Yano, R., Seo, M., 2010. Abscisic acid and the control of seed dormancy and germination. Seed Science Research 20, 55–67.

Negm, F.B., Smith, O.E., Kumamoto, J., 1972. Interaction of carbon dioxide and ethylene in overcoming thermodormancy of lettuce seeds. Plant Physiology 49, 869–872.

Roth-Bejerano, N., Sedee, N.J.A., Van der Meulen, R.M., Wang, M., 1999. The role of abscisic acid in germination of light-sensitive and light-insensitive lettuce seeds. Seed Science Research 9, 129–134.

Saini, H.S., Consolacion, E.D., Bassi, P.K., Spencer, M.S., 1986. Requirement for ethylene synthesis and action during relief of thermoinhibition of lettuce seed germination by combinations of gibberellic acid, kinetin, and carbon dioxide. Plant Physiology 81, 950–953.

Saini, H.S., Consolacion, E.D., Bassi, P.K., Spencer, M.S., 1989. Control processes in the induction and relief of thermoinhibition of lettuce seed germination: actions of phytochrome and endogenous ethylene. Plant Physiology 90, 311–315.

Sarath, G., Bethke, P.C., Jones, R., Baird, L.M., Hou, G., Mitchell, R.B., 2006. Nitric oxide accelerates seed germination in warm-season grasses. Planta 223, 1154–1164.

SPSS Inc., 2008. SPSS 13.0 package for Windows. Chicago, Illinois.

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, N., Iuchi, S., Kobayashi, M., Yamaguchi, S., Kamiya, Y., Nambara, E., Kawakami, N., 2008. High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiology 146, 1368–1385.

Torres, J., Sharpe, M.A., Rosquist, A., Cooper, C.E., Wilson, M.T., 2000. Cytochrome *c*oxidase rapidly metabolises nitric oxide to nitrite. FEBS Letters 475, 263–266.

Vidaver, W., Hsiao, A.I., 1975. Actions of gibberellic acid and phytochrome on the germination of Grand Rapids lettuce seeds. Plant Physiology 53, 266–268.

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